

The Effect of *Saccharomyces Cerevisiae* On in Vitro Rumen Digestibilities of Dry Matter, Organic Matter and Neutral Detergent Fibre of Different Forage: Concentrate Ratios in Diets

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Geliş Tarihi: 03.07.2001

Summary: The aim of this research was to investigate the effect of supplementation of *Saccharomyces cerevisiae* viable yeast culture (Yea-Sacc¹⁰²⁶, 5×10^9 organisms/g) into diets having different forage: concentrate ratio on the in vitro rumen dry matter, organic matter and neutral detergent fibre digestibilities. In this research, four cannulated sheep were used in 4x4 latin square design and fed on 70 % alfalfa hay + 30 % concentrate (Diet I), 70 % alfalfa hay + 30 % concentrate + 4 g Yea-Sacc¹⁰²⁶ (Diet II), 30 % alfalfa hay + 70 % concentrate + 4 g Yea-Sacc¹⁰²⁶ (Diet III) and 30 % alfalfa hay + 70 % concentrate (Diet IV) respectively. The duration of each experimental period was 20 days. On the 20th day, rumen digesta was collected from sheep for in vitro incubation 2 h after morning feeding. At the end of experiment, supplementation of yeast culture on high forage ration significantly increased the in vitro rumen dry matter, organic matter and neutral detergent fibre digestibilities ($P < 0,001$), while did not affect on high concentrate ration.

Keywords: *Saccharomyces cerevisiae*, Dry matter, Neutral detergent fibre, Organic matter, in vitro digestibility

***Saccharomyces cerevisiae*'nin Kaba: Konsantre Yem Oranı Farklı Rasyonlarda in Vitro Rumen Kuru Madde, Organik Madde ve Neutral Detergent Fibre Sindirilebilirliği Üzerine Etkisi**

Özet: Bu araştırmanın amacı farklı kaba: konsantre yem oranına sahip rasyonlara *Saccharomyces cerevisiae* canlı maya kültürü ilavesinin (Yea-Sacc¹⁰²⁶, 5×10^9 organisms/g) in vitro rumen kuru madde, organik madde ve neutral detergent fibre sindirilebilirliği üzerine etkisini incelemektir. Araştırmada rumen kanülü takılmış 4 baş koyun, 4x4 latin kare düzeninde kullanılmış ve sırasıyla %70 yonca kuru otu + %30 konsantre yem, %70 yonca kuru otu + %30 konsantre yem + 4 g Yea-Sacc¹⁰²⁶, %30 yonca kuru otu + %70 konsantre yem + 4g Yea-Sacc¹⁰²⁶ ve %30 yonca kuru otu + % 70 konsantre yemden oluşan rasyonlarla beslenmişlerdir. Araştırmanın her bir periyodu 20 gün sürmüştür. Yirminci günde, koyunlardan in vitro inkübasyon için sabah yemlemesinden 2 saat sonra rumen sıvıları toplanmıştır.

Denemenin sonunda kaba yem ağırlıklı rasyona maya kültürü ilave edilmesi in vitro rumen kuru madde, organik madde ve neutral detergent fibre sindirilebilirliklerini önemli derecede artırırken ($P < 0,001$), konsantre yem ağırlıklı rasyonda etkisi görülmemiştir.

Anahtar Kelimeler: *Saccharomyces cerevisiae*, Kuru madde, Neutral detergent fibre, Organik madde, İn vitro sindirilebilirlik

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Introduction

Human being has used yeast cultures to produce fermentation products such as bread and wine. In addition, yeast cultures are used in human and animal nutrition as protein source. However, as a result of the biotechnological improvements in recent years, live yeast cultures are also used as a feed additive to increase animal production. Yeast cultures used as a feed additive are in the probiotics class which affect indigestion system of animals in good way¹¹.

Recent papers indicate that supplementation of viable yeast culture of *Saccharomyces cerevisiae* into feed ration may increase milk production^{21,26}, weight gain^{13,16}, feed intake^{14,17} and digestion^{25,28} improve anaerobic and cellulolytic bacteria^{8,12}, decrease the ruminal pH¹², and alter the patterns of volatile fatty acids production^{19,22}. In contrast, it was reported that yeast culture did not affect rumen pH²², total volatile fatty acids and rumen ammonia^{12,25}, did not change milk production and composition^{2, 6}, feed intake and weight gain^{18,24} and digestion^{2,12,27}.

It was suggested that *Saccharomyces cerevisiae* used as feed additive in ruminant diets shows its effect by changing the rumen microfauna⁷. Some researchers studied the mechanism of this effect and they suggested that yeast culture helps to create anaerobic milieu by using O₂ in rumen²⁰ and affects the increase and activities of anaerobic bacteria such as *Fibrobacter succinogenes* and *Ruminococcus albus* strain 7⁹, especially important for the digestion of cellulose; and *Megasphaera elsdenii* DSM 20246⁵ and *Selenomonas ruminantium* HD4¹⁵ using lactic acids.

The purpose of this study was to measure the effect of supplementation of viable yeast culture on in vitro dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF) digestibilities in different forage: concentrate ratio diets.

Materials and Methods

1. Animals and Feeding

Four mature rumen cannulated Kivircik sheep were used to provide rumen fluid. All animals were fed on alfalfa hay and concentrate cubes. Sheep were housed in individual pens and

allowed ad-libitum access to water. Sheep were fed on four diets following a random sequence in a 4x4 Latin square design as described by Duzgunes et al.¹⁰. The experimental diets consisted of 70 % alfalfa hay + 30 % concentrate (Diet I), 70 % alfalfa hay + 30 % concentrate + 4 g Yea-Sacc¹⁰²⁶ (Diet II), 30 % alfalfa hay + 70 % concentrate + 4 g Yea-Sacc¹⁰²⁶ (Diet III) and 30 % alfalfa hay + 70 % concentrate (Diet IV). The nutrient contents of diets are shown in Table I.

Table I. Composition and nutrients content of diets

Tablo I. Rasyonların bileşimi ve besin maddesi içerikleri

	Diet I	Diet II	Diet III	Diet IV
Alfalfa hay %*	70	70	30	30
Concentrate%*	30	30	70	70
Yea-Sacc ¹⁰²⁶	-	+	+	-
Dry Matter	88.76	88.86	89.76	89.80
Crude Protein %*	21.51	21.78	20.32	20.28
Ether Extract %*	2.90	2.98	3.66	3.56
Organic Matter %*	88.91	88.99	91.21	91.15
NDF %*	41.96	41.90	35.02	35.10

* Bases of % Dry Matter

* % Kuru Maddede

The duration of each experimental period was 20 days. On the 20th day, rumen digesta was collected from sheep for in vitro incubation 2 h after feeding.

2. Rumen Digesta Sampling and in vitro incubations

The digesta samples of rumen content were obtained manually through the cannula from various sites in the rumen. The digesta sample from each animal was filtered through four layers of gauze cloth and immediately transferred in a flask kept at 39°C.

In vitro study was carried out according to the methods of Tilley and Terry²³. The incubation inoculum was prepared by diluting the digesta inoculum with the buffer in a 1:4 (vol/vol) ratio. The four inocula were incubated with diet samples similar to the diet of the donor animals (Table I). A half-gram diet sample was placed into 50 ml sterile plastic tube and then 40 ml inoculum was added into each tube. The tubes were incubated for 48 h at 39°C. Tubes were gently swirled by hand once time every 6 h. Eight

replications per diet were applied for inoculum obtained from each donor animal in this study.

At the end of the 48 h incubation period, the tubes were centrifuged at 6000 rpm for 15 min., and supernatant was discarded. Forty ml of 0.2 % pepsin + 0.1 N HCl was added into tubes. The tubes were reincubated for an additional 48 h. At end of this period, the tubes were recentrifugated at 6000 rpm for 15 min., and supernatant was discarded. Forty ml of pure H₂O was added in tubes and were recentrifuged to wash out the residual acid. The tubes containing pellets were dried in an air forced oven at 60°C for 48 h to determine the residual DM weights¹. All pellets were pooled in a tube and analyzed for OM, NDF¹.

Data were analyzed using a model for 4x4 Latin square design. When main effect means were significant, treatment means were tested statistically using Tukey's test¹⁰.

Results

The chemical composition of diets is shown in table I. The in vitro DM, OM and NDF digestibility values are given in table II. From table II, it was found that DM, OM and NDF digestibilities of Diet III and IV had a significant increase compared with Diet I and II (P<0.001). The digestibility of DM, OM and NDF in Diet II was significantly higher than in Diet I (P<0.001).

In our study, it was determined that the addition of *Saccharomyces cerevisiae* live yeast culture into high forage ration increased significantly the digestibility of dry matter in vitro (P<0.001). However, the addition of *Saccharomyces cerevisiae* live yeast culture into high concentrate ration had not a significant effect on the digestibility of dry matter in vitro. Difference between high forage ration and high concentrate ration was found statistically important (P<0.001). The reason of this importance was because of high concentrate in Diet III and IV. Likewise, Zelenak et al.²⁸ reported that the addition of *Saccharomyces cerevisiae* live yeast culture into high concentrate ration increased the digestibility of dry matter in vitro even it is not statistically important. However, Carro et al.³, Chiquette⁶, Harrison et al.¹² and Wholt et al.²⁷ noticed that *Saccharomyces cerevisiae* live yeast culture did not affect the digestibility of apparent dry matter.

In our experiments, it was determined that the addition of *Saccharomyces cerevisiae* live yeast culture into high concentrate ration did not significantly affect the digestibility of organic matter in vitro (Diet III and IV). However, the addition of *Saccharomyces cerevisiae* live yeast culture into high forage ration increased significantly the digestibility of organic matter in vitro (P<0.001). In addition, in terms of the digestibilities of organic matter in vitro, there was no difference between high forage ration and high

Table II. Effect of *Saccharomyces cerevisiae* in diets on in vitro digestibilities

Tablo II. Rasyonların in vitro sindirilebilirliğine *Saccharomyces cerevisiae*'nın etkisi

% Digestibilities	Diet I 70 % alfalfa hay 30 % concentrate \bar{x} S \bar{x}	Diet II 70 % alfalfa hay 30 % concentrate Yea-Sacc ¹⁰²⁶ \bar{x} S \bar{x}	Diet III 30 % alfalfa hay 70 % concentrate Yea-Sacc ¹⁰²⁶ \bar{x} S \bar{x}	Diet IV 30 % alfalfa hay 70 % concentrate \bar{x} S \bar{x}
Dry Matter	64.54 ^a 0.33	66.36 ^b 0.47	74.21 ^c 0.40	75.23 ^c 0.42
Organic Matter	62.74 ^a 0.35	65.18 ^b 0.29	73.31 ^c 0.42	74.39 ^c 0.43
NDF	42.07 ^a 0.48	45.28 ^b 0.41	51.06 ^c 0.54	51.70 ^c 0.49

a-c: significantly different from each diet in the same row (P<0.001)

a-c: aynı satırda farklı harfi taşıyan rasyonlar arası farklar önemlidir (P<0.001).

Discussion

In vitro dry matter, organic matter and NDF digestibility values belonging to Diets I, II, III and IV were given in Table II.

concentrate ration. In some in vivo studies done in this subject, it was reported that *Saccharomyces cerevisiae* live yeast culture did not affect the digestibility of organic matter^{3,6}.

An increase was observed in the in vitro digestibility of NDF by adding yeast into high forage ration. Nevertheless, the addition of yeast

into high concentrate ration did not influence the in vitro digestibility of NDF. In terms of the in vitro digestibility of NDF, difference between high forage and concentrate rations was found significantly important ($P < 0.001$). Similarly, Zelenak et al.²⁸ reported that the addition of yeast into not only forage but also concentrate rations increased the in vitro digestibility of NDF even the difference was not statistically important. In addition, Zelenak et al.²⁸ suggested that hemicellulose digestibility was significantly higher after *Saccharomyces cerevisiae* supplementation in both high forage and concentrate diets. However, in the apparent digestibility studies done in this subject, it was reported that *Saccharomyces cerevisiae* live yeast culture did not have any important effect on the digestibility of NDF^{3,6,12,27}.

Many researchers noticed that *Saccharomyces cerevisiae* live yeast culture shows its effect clearly in high concentrate rations^{4,7}. However, in the present study, when *Saccharomyces cerevisiae* live yeast culture did not affect the digestibility of high concentrate ration, it increased the digestibility of high forage ration. It was thought that the reason of this situation could be the stimulation of yeast culture on the number and activities of total anaerobic and cellulolytic bacteria.

It was concluded that *Saccharomyces cerevisiae* live yeast culture could increase the digestibilities of dry matter, organic matter and NDF in high forage rations.

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